Histopathologic effect of chronic use of sildenafil citrate on the choroid & retina in male rats

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Background & objectives: Sildenafil citrate is an oral medication used to treat male impotence by the inhibition of phosphodiesterase-5 in the corpus cavernosum and subsequent facilitation of penile erection. Though the ocular side effects of sildenafil have been reported, no information is available on the histopathologic effects of chronic use of sildenafil citrate on the ocular vasculature. The present study was undertaken to study the histopathologic effects of chronic use of sildenafil on the retina and choroid of male rats.

Methods: Twelve adult male Wistar rats were used in the study. Six of them were given 8 mg/kg/day sildenafil citrate orally on alternate days, the other six rats were used as control. The animals were sacrificed after 4 wk of treatment, and the eyes were fixed in 10 per cent formalin solution and sectioned after embedding in paraffin. Sections were cut, stained with haematoxylin-eosin (HE) or periodic acid Schiff (PAS) and examined under light microscope. The choroidal capillary diameter was also measured.

Results: The choroidal capillaries were more dilated in the sildenafil citrate treated group (mean capillary diameter 3.44 ± 1.68 µm versus the control of 1.78±1.36 µm, \( P<0.001 \)). The retinal layers and their configuration were unchanged in both the groups.

Interpretation & conclusion: Chronic use of sildenafil citrate can cause dilatation and congestion in the choroidal vasculature of male rats.

Key words Choroid vasculature - sildenafil citrate - wistar rat

Sildenafil citrate is an effective drug for erectile dysfunction due to organic causes1-3, however, side-effects have been reported such as flushing, headache, and congestion4,5. Sildenafil is a selective inhibitor of the cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase (PDE)-5. It has a mild inhibitory effect on PDE-6 (about 10-fold lower affinity than for PDE-5), which is located in the retinal photoreceptors and regulates the levels of cGMP in the retina5,6. Sildenafil is therefore potentially retinotoxic due to an associated increase in retinal c-GMP, and it has been shown to cause a depression of electroretinogram (ERG) functions suggesting clinical toxicity of the retina7-9. In addition to the inhibitory effect on PDE in the retina, sildenafil may also alter choroidal blood flow due to its systemic effects on vascular smooth muscle. Ocular side-effects of sildenafil have been frequently reported, generally in terms of changes in colour vision and light flashes10. It is thus
possible that sildenafil changes ocular circulation\textsuperscript{11,12}. However, sildenafil citrate is still considered to be a safe drug despite reports of its ocular side-effects. To the best of our knowledge, there are no reports on the histopathological changes in the eye vasculature following chronic sildenafil use. Therefore, the present study was undertaken to investigate the histopathological effects of chronic use of sildenafil citrate on the choroid and retina of male rats.

**Material & Methods**

The study was performed in the Department of Histology and Embryology at the University of Celal Bayar, Manisa, Turkey. The Ethics Committee at Celal Bayar University, School of Medicine, approved the study protocol.

Twelve fertile adult male Wistar rats were used in the study. The animals were fed a standard laboratory rat diet, and six were given 8 mg/kg/day sildenafil citrate orally on alternate days (three days in a week) for 4 wk\textsuperscript{13}, the remaining six rats were left untreated and served as controls. The animals were sacrificed after 4 wk of treatment, and the eyes were dissected out and fixed in 10 per cent of formalin solution following treatment in 95 per cent ethanol for 24 h and then in chloroform for 5 h. The eyes were embedded in paraffin wax and 5 µm serial sections were taken. Random sections were selected and stained with either haematoxylin and eosin (Sigma, USA) (H-E) or with periodic acid Schiff (PAS) (Sigma, USA) according to the recommended routine protocols\textsuperscript{14,15}. The slides were mounted using entellan (Surgipath, MX-004) and covered with glass coverslips prior to viewing under the Olympus BX-40 light microscope (Tokyo, Japan).

To estimate the diameter of the choroidal capillaries, the H-E stained serial sections were recorded using a video camera (JVC) which was attached to the light microscope, and then downloaded to a computer for the measurements. The diameter of the capillaries was measured across capillary lumen. For each capillary, at least four measurements were taken. Two independent observers, blinded to the sample origin, recorded and evaluated the measurements. The data were analysed statistically using Mann-Whitney U test, $P$ value < 0.05 was considered significant.

**Results**

Dilatation and congestion of the choroidal capillaries was observed in the sildenafil treated group compared to controls (Fig. 1A-D). The mean capillary diameter being 3.44±1.68 µm in the sildenafil treated group and 1.78 ± 1.36 µm in the control group ($P$<0.001). Despite this dilatation of the choroidal capillaries in the treated group, the basement membranes of the capillaries in the choroid were seen as continuous sheets, same as in controls (Fig. 1C-D).

When the other anatomical areas of the eyes were compared under the light microscope, no histological differences were detected between controls and the treated group (data not shown). No histopathological changes were seen in the retina of the control (Fig. 2-A) or the treated (Fig. 2-B) groups.

**Discussion**

Sildenafil citrate is shown to be effective in treating erectile dysfunction by selectively inhibiting the cGMP-specific PDE-5 in the corpus cavernosum smooth muscle cells\textsuperscript{16}. It also weakly inhibits PDE-6, which is present in high concentrations in the cone and rod cells and plays a key role in the light signal phototransduction of the retina. The most common adverse effects associated with sildenafil are headache, flushing and dyspepsia\textsuperscript{17}, the ocular side-effects include altered vision, mainly involving changes in colour, hue or brightness perception\textsuperscript{12,18}. Cardiovascular deaths and retinal vascular events in individuals taking sildenafil have also been reported\textsuperscript{19,20}. However, some investigators supported that PDE-6 inhibition causes the dose-dependent clinical effects of visual disturbance in men taking sildenafil and the implications of the long-term daily use of sildenafil in men are not clear\textsuperscript{11,21}.

The results of the present study demonstrated that sildenafil citrate can cause dilatation of the choroidal capillaries in the rat, although the retina was not
seen to be affected histopathologically. Thus the sildenafil has its major effect on the choroidal circulation in male rats, suggesting the potential involvement of sildenafil in circulatory defects of the choroid in humans.

Toxicity studies on dogs\textsuperscript{22} with a high dose (60 mg/kg) of sildenafil over extended periods have not shown changes in the retinal architecture on histological examination. Therefore, it may not be possible to show histologically the retinotoxicity of chronic use of sildenafil. Our study also did not reveal any changes in the retinal morphology in the eyes of rat. However, choroidal dilatation was found and it may cause choroidal disfunction. In addition to effects on PDE in the retina, there is a possibility that sildenafil may alter the vascular flow or choroidal volume because of its systemic effects on vascular smooth muscle. Birch \textit{et al}\textsuperscript{23} reported no significant changes in intraocular pressure after acute treatment with sildenafil citrate, but choroidal diameter or volume were not measured. Our results show that there is a dilatation of the choroidal vessels after sildenafil treatment and this may lead to congestive effects within the eye. An increase in choroidal vessels volume and diameter may also
affect retinal and retinal pigment epithelial functions and, if severe, could predispose to retinal detachment, or oedema, or even to glaucoma.

The results of the present study demonstrate that chronic use of sildenafil citrate causes complications in the choroid of the rat. These visual effects need to be investigated in humans.

References


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